a green center. The same percentage of colorless colonies developed after the application of pressure for 2 hours as after application for 20 minutes.

Of the cells streaked on agar plates after exposure to 500 atm for ½ hour or 2 hours, only 1 percent showed color mutation, indicating that the phenomenon is pressure-dependent though apparently not time-dependent. Further study is required however, before definitive conclusions can be drawn. Although the mutant colonies appeared white on agar, they appeared yellow-orange when grown in liquid, thus reflecting synthesis of carotenoids (*10*).

Two additional "pressure" mutants, designated PR-2 and PR-3, were isolated. Mutant PR-2 synthesized about twice as much carotenoid as did PR-1 or PR-3 on the basis of the dry weight of the cells. This relationship is based on the $E^{1\%}_{1\text{-cm}}$ (*11*) value of 2500 for β-carotene (*12*) measured from the absorption spectrum of the total carotenoids extracted in 95 percent ethanol. The carotenoids have not been isolated or identified.

Neither in Cattell's review (*13*) nor in the studies cited by Johnson, Eyring, and Polissar (*14*) are there references to pressure-caused mutations. Whether the effect reported here is a reflection of a chromosomal or cytoplasmic gene change cannot be stated with certainty. However, there is evidence which suggests a lack of direct nuclear control over chloroplast formation. Furthermore, several studies show that both DNA and RNA are present in chloroplasts (*15*). Such evidence favors cytoplasmic mutation, rather than chromogenic mutation, as the probable mechanism upon which hydrostatic pressure acts. Also, Hedén's studies (*16*) suggest that nucleic acids are probably targets of hydrostatic pressure. It can be postulated that pressure acts on a stage of chloroplast formation in which there is an increase in volume (*14*) of either the structural organelle or some molecular phase vital to its development.

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References and Notes

1. S. H. Hutner and L. Provasoli, in *Biochemistry and Physiology of Protozoa*, A. Lwoff, Ed. (Academic Press, New York, 1951), vol. 1.
2. L. Ebringer, *Nature* **18**, 606 (1961).
3. J. A. Gross, T. L. Jahn, E. Bernstein, *J. Protozool.* **2**, 71 (1955).
4. E. G. Pringsheim and O. Pringsheim, *New Phytol.* **51**, 65 (1952).
5. M. DeDeken-Grenson, *Arch. Intern. Physiol. Biochim.* **67**, 506 (1959); H. Lyman, H. T. Epstein, J. A. Schiff, *J. Protozool.* **6**, 264 (1959).
6. Obtained from Dr. J. J. Wolken, Biophysical Research Laboratory, University of Pittsburgh Medical Center, Pennsylvania.
7. C. L. Greenblatt and J. A. Schiff, *J. Protozool.* **6**, 23 (1959).
8. Pressure vessels were fabricated by the Experimental Mechanics and Materials section of IIT Research Institute from 20-cm heat-treated round steel stock, from high strength steel forgings, or by modifying Mark II 40-cm naval projectiles. Bores range from 10 cm to 40 cm; pressures can be controlled from ambient up to 10,200 atm in vessels of 10-cm bore, and up to 1080 atm in vessels of 40-cm bore.
9. J. A. Gross, S. K. Wirtschafter, E. Bernstein, T. W. James, *Trans. Am. Microscop. Soc.* **75**, 480 (1956); R. A. Olson and E. K. Engel, *Brookhaven Symp. Biol.* **11**, 303 (1958).
10. J. A. Gross, paper presented at Annual Meeting, Midwest Sect., American Society of Plant Physiologists, University of Chicago, 11 June 1964.
11. $E^{1\%}_{1\text{-cm}}$ is the extinction (optical density) of a 1-percent solution measured through a 1-cm light path.
12. T. W. Goodwin and J. A. Gross, *J. Protozool.* **5**, 292 (1958).
13. McK. Cattell, *Biol. Rev. Cambridge Phil. Soc.* **11**, 441 (1936).
14. F. H. Johnson, H. Eyring, M. J. Polissar, *The Kinetic Basis of Molecular Biology* (Wiley, New York, 1954).
15. T. Iwamura, *Biochim. Biophys. Acta* **61**, 472 (1962); E. H. L. Chun, M. H. Vaughan, Jr., A. Rich, *J. Mol. Biol.* **7**, 130 (1963); A. B. Jacobson, H. Swift, L. Bogorad, *J. Cell Biol.* **17**, 557 (1963); A. Gibor and S. Granick, *Proc. Natl. Acad. Sci. U.S.* **50**, 1164 (1963); C. J. Pollard, *Arch. Biochem. Biophys.* **105**, 114 (1964); A. Gibor and S. Granick, *Science* **145**, 890 (1964).
16. C-G. Hedén, *Bacteriol. Rev.* **28**, 14 (1964).
17. I thank Dr. R. Cornish and associates, Experimental Mechanics and Materials Section, IIT Research Institute, in whose laboratory the high-pressure exposures were performed. This research was supported by IIT Research Institute under Projects L1000L1 and L1002.

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Complement and Hemolytic Antibody: Changes in Their Activity Induced by Mercaptoethanol

Abstract. *Cell-bound complement components are not destroyed by mercaptoethanol. Destruction of cell-bound hemolytic antibody was prevented by cell-bound complement components. In the absence of cells, the complement components C'3 and C'4 were most susceptible to destruction by mercaptoethanol; C'1, C'1a, and C'2 were most resistant. Mercaptoethanol (0.01*M*) increases C'1 activity, and this increase is not due to activation of C'1 to C'1a. Destruction of some components required both mercaptoethanol and iodoacetamide treatment.*

Studies on the effects of a mild reducing agent on the reactants which participate in cytotoxic reactions mediated by specific antibody and complement (C′) are described in this report. Specifically, we have examined the effects of mercaptoethanol (MSH) or the effects of MSH and alkylation by iodoacetamide (IA) on C′, on its components, on rabbit antibody (A) to the boiled stromata of sheep erythrocytes (E), and on the cellular intermediate products leading to hemolysis. The reaction of A with E occurs at discrete sites (S) to produce SA (*1*).

More than 95 percent of the hemolytic activity of antiserums prepared against boiled stromata of E is due to antibody with a molecular weight of approximately 10^6 (β_2M- or 19*S*-globulin). This rabbit antibody, A, exposed to mild reduction loses its ability to sensitize E to the hemolytic action of C′ (*2*).

Sources and preparation of guinea pig C′, E, A, veronal buffers (VBS), and low ionic strength buffers, as well as methods for preparing cellular intermediates and for measuring A, C′, and C′ components have been described (*3*). These reagents were treated with two different concentrations of MSH (Table 1) at 37°C for 30 minutes at *p*H 7.4. Solutions of IA (Table 1) were added, where needed, at room temperature. During addition of IA the *p*H was maintained between 6 and 8 by adding NaOH (0.2 to 0.5*M*) as needed. Before testing for their effects on the reagents, MSH and IA were removed either by dialysis (for fluid phase reagents) or by centrifuging and washing (for reagents bound to cells).

First the effect of MSH on fluid phase C′ and its components was investigated. When whole guinea pig serum was exposed to 0.01*M* MSH, C′ activity was slightly decreased in some experiments, and in others it was unchanged. In all cases there was a marked increase in C′1 activity, and in some experiments the C′1 activity was doubled. When treatment by 0.01*M* MSH was followed by addition of IA, the activities of both C′1 and the C′3 complex were drastically lowered. These losses are thought to account for the marked decrease in activity of whole C′ after treatment with MSH and IA (Table 1, lines 1, 2, and 4). When the MSH concentration was increased to 0.05*M* and IA was not added, both

C'4 and the C'3 complex were inactivated (Table 1, lines 4 and 5); C'1 was still unaffected. When 0.05*M* MSH was followed by IA, C'1 activity was also eliminated (Table 1, line 2).

In contrast to the behavior of C'1 in whole serum, the activity of purified, activated C'1 (C'1a) is unaffected by treatment with 0.01*M* MSH and is only slightly decreased by MSH followed by IA (Table 1, line 6). This difference in behavior between C'1 and C'1a will be discussed.

Both native and purified C'2 are equally resistant to MSH (Table 1, lines 3, 7, and 8).

Next, the effects of MSH and IA on the cellular intermediates were tested. Treatment of E with 0.05*M* MSH with or without IA had no effect on the susceptibility of E to lysis by A and C'. On the other hand, EA similarly treated were not hemolyzed by C' (Table 1, line 9). Destruction of A on EA by MSH can be prevented by converting all SA to SAC'1a before treatment with MSH. After treatment of EAC'1a with MSH, C'1a can be removed, and the product EA is fully susceptible to lysis by C' (Table 1, line 11).

The effect of MSH on EAC'4 (a cellular intermediate obtained by removing C'1a from EAC'1a,4) was tested. The following experiments show that the intermediate product SAC'4 is resistant to MSH whereas SA on the same cell are not. The total number of sites per EAC'4 capable of reacting with and transferring C'1a before treatment of EAC'4 with MSH was 225 and after treatment it was 52 (Table 1, line 12). To determine whether this decrease was due to destruction of SAC'4 or SA or both, a portion of EAC'4 which had been exposed to MSH was treated with C'1a to convert all sites to SAC'1a,4. A preparation of EAC'1a,4 was also made from an untreated portion of EAC'4. These two preparations of EAC'1a,4 had the same number of SAC'4 per cell, for each had the same t_{max} (*4*). From the t_{max} determination, the number of SAC'4 per cell was calculated to be about 60 (Table 1, line 13). Thus the loss of sites on EAC'4 capable of transferring C'1a must have been due to destruction of SA by MSH.

We have also found that MSH or MSH followed by IA has no effect on either the decay rate of SAC'4,2a or on the reactivity of SAC'4,2a with the C'3 complex [in the form of C'-EDTA (ethylene diaminetetraacetate)] (Table 1, lines 14 and 15). In addition, the intermediate product E* (stabilized in 0.09*M* EDTA) was not affected by MSH treatment (Table 1, line 16).

Table 1. Effects of MSH (0.01*M* and 0.05*M*) and MSH followed by IA (0.01*M* and 0.05*M*) on C', its components, and on cellular intermediates. The relative titers of C', C'3, and A are expressed in reciprocal dilution needed to lyse 50 percent of cells. The rest are expressed in effective molecules per cell (M/cell) or in percentage of lysis as indicated.

No.	Substance	VBS	0.01*M* MSH		0.05*M* MSH		0.05*M* IA
			Alone	0.01*M* IA	Alone	0.05*M* IA	
1	Whole $C'H_{50}$	114	78	18	<10	<10	88
2	C'1 (M/cell)*	4,700	7,010	570	4,540	42	4,900
3	C'2 (M/cell)*	6,400	7,200	5,114	4,026	1,647	7,600
4	C'3 (C'EDTA) C'3 H_{50}*	750	510	40	5	0	1,000
5	C'4 (M/cell)*	17,000	17,400	18,800	420	940	17,500
6	Purified C'1a (M/cell)	30,000	27,400	20,300	24,900	17,800	32,000
7	Purified C'2 (M/cell)	540	290	290	250	160	545
8	Purified C'2 (M/cell)	640	550		560		
9	EA (A H_{50})	32,000			<2,000	<2,000	31,000
10	EA + C'2 (A H_{50})	32,000			<2,000	<2,000	31,000
11	EAC'1a (A H_{50})	32,000			26,000	~3,000	31,000
12	EAC'4 (total SA/cell including SAC'4)†	225			52		
13	EAC'4 (total SAC'4/cell)‡	60			60	<5	
14	Rate of decay of SAC'4,2a, min^{-1}	0.047			0.047	0.047	0.045
15	EAC'4,2a, % lysis with C'EDTA	77			79	76	75
16	E*, % lysis	96			88		

* Contained in whole C'. † As determined by the ability of EAC'4 to react with and transfer C'1a. ‡ As determined by t_{max} measurement (*4*).

In contrast, the presence of C'2 during exposure of EA to MSH did not protect SA against the destruction of MSH (Table 1, line 10).

Two major points emerge from these observations. The first is that C'1 in the native state and partially purified C'1a differ in their susceptibility to MSH. To explain the increase in C'1 activity by treatment with 0.01*M* MSH we considered the hypothesis that mild reduction by MSH may activate C'1 to C'1a. However, some experiments indicated that native C'1 treated with MSH was still in the precursor state. Therefore a more likely possibility is that treatment with 0.01*M* MSH removes an inhibitor which does not play a role in the conversion of C'1 to C'1a.

The destruction of C'1 by MSH treatment followed by IA (this combination has little effect on C'1a) could mean that mild reduction followed by alkylation permanently inhibits the conversion of C'1 to C'1a; once activation has taken place, bonds in the C'1 molecule which can be reduced and alkylated are no longer available in the C'1a molecule.

The second major point is that the susceptibility of A and C' components to MSH treatment may change depending upon whether the reagents are in the fluid phase or are fixed to the cell surface. We have shown that C'1a protects A in the complex SAC'1a. We have also shown that after fixation of C'4 by SAC'1a, C'4 is no longer susceptible to destruction by 0.05*M* MSH. The observation that EAC'4 treated with MSH contained SAC'4 reactive with C'1a was interpreted to mean that both C'4 and A in this complex are protected from destruction by MSH. This interpretation should be viewed with caution since it is conceivable that once C'4 is fixed A plays no further role in maintaining a hemolytically active site. However, until evidence is forthcoming to show that C'1a can react with the cell surface in the absence of A, we prefer the above interpretation.

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References and Notes

1. Terminology conforms to that recommended in H. J. Rapp and T. Borsos, *Science* **141**, 738 (1963).
2. K. Amiraian and B. Ferris, *Federation Proc.* **23**, 558 (1964).
3. E. A. Kabat and M. M. Mayer, *Experimental Immunochemistry* (Thomas, Springfield, Ill., ed. 2, 1961); H. J. Rapp and T. Borsos, *J. Immunol.* **91**, 821 (1963); T. Borsos and H. J. Rapp, *ibid.*, p. 851; T. Borsos, H. J. Rapp, U. L. Walz,, *ibid.* **92**, 108 (1964); M. M. Frank, H. J. Rapp, T. Borsos, *ibid.* **93**, 409 (1964).
4. The time, t_{max}, at which SAC'1a,4,2a production in the reaction between EAC'1a,4 and C'2 reaches its maximum is dependent only on the number of SAC'1a,4 per unit volume and is independent of C'2 concentrations. In comparing the t_{max} of two different EAC'1a,4 preparations, the cell concentration, ionic environment, and temperature at which the determinations are performed must be the same. See T. Borsos, H. J. Rapp, M. M. Mayer, *J. Immunol.* **87**, 310 (1961).

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